APPLICATION OF HIGH CELL DENSITY AIRLIFT BIOREACTORS TO BIO-ETHANOL PRODUCTION – STUDY ON OPTIMAL BIOREACTOR OPERATION

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ABSTRACT: In this paper a hydrodynamic and rheological analysis of a continuous airlift bioreactor with high cell density system is presented. A highly flocculating recombinant strain of *Sacharomyces cerevisae* containing genes for lactose transport (lactose permease) and hydrolysis (β-galactosidase) was exploited to ferment lactose from cheese whey to ethanol. The magnetic particle-tracer method was used to assess the effect of operational conditions (air flow rate, biomass concentration) on hydrodynamic behaviour of an the airlift bioreactor during the fermentation process. Measurements of liquid circulation velocity showed the existence of a critical value of biomass concentration at which a dramatic deceleration of net liquid flow appeared with increasing biomass quantity. Rheological analysis revealed dramatic changes in viscosity of the yeast floc suspension at the same biomass concentration of about 73 $g.dm^{-3}$ corresponding to 42.8 % v/v of solid fraction. These facts have a particular importance for the successful processing of high cell density airlift bioreactor as only a circulated flow regime will be favourable to keep the solid particles in suspension state and evenly distributed throughout the bioreactor.

Keywords: bio-ethanol, continuous reactor, biomass characteristics

1 INTRODUCTION

In recent years, there has been a growing interest in bioreactors utilising immobilised enzymes and cells in order to improve the bioprocess productivity. In fact, there are many specific advantages of the immobilised systems in comparison with the more conventional systems, in which the bio-agents are suspended freely (cells) or dissolved in a bulk aqueous medium (enzymes) [1]. In addition to conventional immobilization techniques, the use of flocculating microorganisms is very attractive since there is no need of mechanical devices or supports [2, 3].

In practice, a high cell density system (HCDS) usually represents a three-phase (gas-liquid-solid) dispersion operating with a liquid phase in continuous mode. Due to the very high solid loading (up to 50-60 % v/v) usually required [4], maintaining the solid particles suspended and evenly distributed is a major task for successful bioreactor operation. For these systems, a continuous airlift bioreactor (ALR) with an enlarged head zone seems to be a very attractive option due to a high retention of the solid phase and also due to the advantageous combination of sufficient mixing and low shear stress. In airlift bioreactors the maintenance of a solid phase in movement is possible due to the net circulation flow established between the riser and down comer sections. Basically, three flow regimes in three-phase airlift reactors were defined [5]: the packed bed regime, the fluidised bed regime and the circulated bed regime (with the solid phase distributed throughout the reactor).

For high cell density systems, the circulated flow regime is highly desirable providing high gas hold-up, high contacting efficiency between all phases and an even solid distribution throughout the ALR [5]. A collapse of net circulation (bioreactor stalling) would have very negative effects on the overall functionality and productivity of the fermentation. This justifies the need to avoid the bioreactor stalling by the application of an optimal bioreactor design and suitable operating conditions (circulation velocity, gas flow rate, biomass concentration).

One of the attractive possibilities for the utilization of a high cell density system is alcoholic continuous fermentation of lactose from cheese whey using a flocculating yeast. Cheese whey, as a by-product of dairy industry, represents a significant environmental problem due to very high values of BOD and COD. For this purpose, a flocculating recombinant strain of *Sacharomyces cerevisae* containing lactose metabolism genes coding for lactose permease and β-galactosidase was developed, enabling the hydrolysis of lactose to galactose and glucose, followed by sugar conversion into ethanol [6].

The main goal of this work is to study the hydrodynamics of a continuous airlift bioreactor during fermentation of lactose to ethanol using a highly flocculating yeast. The magnetic particle-tracer method was used for the assessment of the effect of operating conditions (air flow rate, biomass concentration) on the hydrodynamics of the airlift bioreactor as a whole and also on its individual sections. In view of the very strong variations observed in circulation velocity during the fermentation process, a rheological analysis of the floc suspensions at different concentrations was also performed and the results correlated with those from the hydrodynamic study.

2 MATERIALS AND METHODS

2.1 Yeast strain and conditions of cultivation

A recombinant *Saccharomyces cerevisiae* NCYC869-A3/T1 flocculent strain expressing the LAC4 (coding for β-galactosidase) and LAC12 (coding for lactose permease) genes of *Kluyveromyces lactis* was used. The construction of the recombinant strain was described by Domingues et al. [6].

The composition of the semi synthetic medium used for cultivation and fermentation was as follows (in g.dm^{-3}): Lactose, 50; KH_2PO_4, 5; $(NH_4)_2SO_4$, 2; $MgSO_4.7H_2O$, 0.4 and yeast extract, 1.

2.2 Bioreactor and operational conditions

The bioreactor used in this work was a 6 L internal loop airlift with an enlarged degassing zone, made of Plexiglas. The head zone had the shape of a reversed cut cone with a cylindrical overhead. The conical section formed a 50° angle with the main body of the reactor. The reactor drawing with all dimensions is presented in Fig. 1. The diameter of the draft tube was 42/50 mm, and the A_D/A_R ratio was 1.36. The air injection was made via a perforated plate of 16 mm diameter, with 10 holes of 0.5 mm diameter each, placed 25 mm below the bottom of the draft tube. The wash-out of biomass was minimised by means of an efficient sedimentation barrier placed around the outlet.

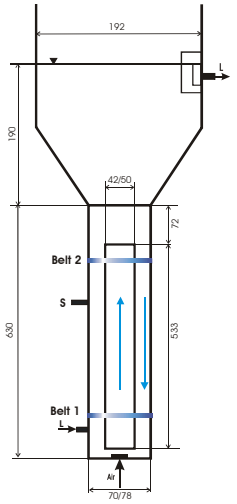

Figure 1. Scheme of a 6 dm^3 continuous airlift bioreactor. Symbols: S - sample port, L - feed and outlet. Belts 1 and 2 indicate the position of the measuring coils for liquid velocity measurement by the magnetic tracer method.

The temperature inside the reactor was maintained at 30±0.5 °C. The pH control was performed by the automatic addition of ammonia solution being the set point fixed at 4±0.1. The reactor was operated at various dilution rates from 0.08 to 0.4 h^{-1} and at aeration rates from 0.63 to 2.76 l.min^{-1} (referred to 20 °C and 1 atm), corresponding to values of 0.1 to 0.45 vvm (normal volume of air per volume of reactor per minute) The air flow rates were controlled by a rotameter and filtered by a microbiological filter.

2.3 Off-line analysis methods

Gravimetry was used to monitor the dry weight cell concentration. It was shown in a previous work [7] that it is reasonable to assume an even distribution in both riser and down comer sections for suspensions of low-density particles (e.g. alginate beads, yeast flocs). Thus, the biomass concentration measured in the down comer was used in the hydrodynamic analysis of the ALR as a representative value of the total biomass. Since the net circulation flow takes place only in the main vertical reactor sections, the biomass present in the head enlarged zone was not considered for the total biomass concentration, c_X.

2.4 Hydrodynamic measurements

A magnetic tracer method [8] was used to determine the liquid velocity in the airlift reactor. The method makes use of the principle of a magnetic metal locator and a flow-following technique. The measuring technique allows liquid velocities, circulation velocity and residence times of the tagging particle to be determined in the airlift reactor, both in its individual sections and as average values for the whole reactor. The overall circulation velocity (V_{LC}) was determined as the average velocity in the two main reactor sections (riser and down comer) assuming that the tagged particle does not reside in the head separator zone.

2.5 Rheological measurements

The viscosity of the floc suspension at different biomass concentrations was measured by means of a Modular Compact Rheometer Physica, MCR-300 (Paar-Physica). Controlled shear-stress measurements were done using parallel-plate geometry of 25 mm diameter, with a gap of 2 mm, at a constant temperature of 30 °C. The applied shear stress ranged from 0.1 to 200 Pa.

3 RESULTS AND DISCCUSION

3.1 Hydrodynamics in airlift bioreactor

The particle with high relative magnetic permeability was aseptically inserted into the bioreactor at the beginning of the fermentation, thus becoming available for velocity measurements at any process time. As the biomass accumulated during the whole fermentation, hydrodynamic measurements were carried out to obtain information on how circulation velocity varies with biomass concentration. Moreover, short-term changes of air flow rate were applied to determine its effect on bioreactor hydrodynamics. As expected, the circulation velocity increased with gas superficial velocity U_{Gc} following a common logarithmic curve [9]. It is evident from the graph that the increase of gas flow rate improves the net circulation flow up to a biomass concentration of ca. 80 g.dm^{-3}.

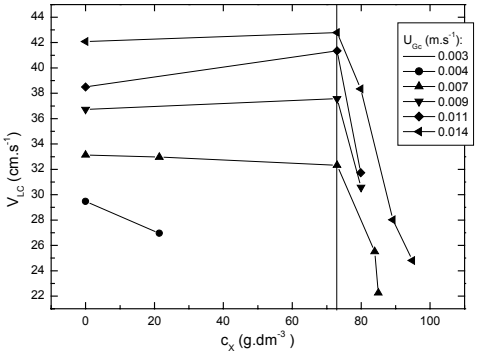

Figure 2. Effect of biomass concentration, c_X, on overall circulation velocity, V_{LC}, at different superficial gas velocity, U_{Gc}. A solid line marks a critical value of biomass concentration, c_{Xcrit}. A range of U_{Gc} applied corresponds to the range of air flow rates Q_G from 0.1 to 0.45 vvm.

The effect of biomass concentration on hydrodynamics during the fermentation process is shown in Fig. 2. The results revealed that the dependence of the overall circulation velocity on biomass concentration does not present a monotonous behaviour for the tested concentration values. Surprisingly, as the biomass accumulates, the velocity V_{LC} remains approximately constant for any of the studied values of air flow rate. This initial plateau on the velocity-biomass concentration curve at lower c_X concentrations was followed by a sudden steep decline indicating a strong degradation of net circulation flow. A dramatic decrease of liquid velocity beyond the critical biomass concentration c_{Xcrit} equal to 73 g.dm^{-3} (corresponding to 42.8 % v/v of solids fraction, see solid vertical line in Fig. 2) was found for all air flow rates applied (e.g. from 73 to 80 g.dm^{-3} the V_{LC} decrease was 20 % on average). This breakpoint was found to be a crucial parameter for the operation of a high cell density airlift bioreactor, representing the onset of potential appearance of bioreactor stalling (stopping the net circulation).

There was also an attempt to experimentally determine the maximum biomass concentration allowing the operation of the airlift bioreactor in a circulated three-phase flow regime. During the whole fermentation no natural regulation of biomass concentration inside the bioreactor was observed. The system continuously accumulated biomass up to a point where reactor stalling occurred. The biomass started to accumulate at the bottom part of down comer, finally resulting in the break-up of the net circulation. The maximum concentration of biomass achieved in the bioreactor before stalling occurred (c_{Xmax}) was found to be higher than the critical value previously defined (e.g. at the highest air flow rate of 0.45 vvm, c_{Xmax} was higher than the critical value by 40 %).

It is worth to notice another important ocurrence observed from the velocity curves in Fig 3: the critical value of biomass concentration was independent of the gas flow rate. It means that on one hand an increase of air flow rate will increase circulation flow rate but on the other hand it will not help to increase the critical limit for biomass concentration for which the strong decrease of the circulation velocity can start to be observed.

3.2 Rheology of floc suspensions

One of the possible reasons of such sudden decrease in circulation velocity with biomass concentration could be a change of viscosity of the yeast floc suspension. Hence, a rheological analysis of floc suspension in the whole range of biomass concentrations was carried out and an appropriate rheological model was suggested to describe the viscosity curves. The Cross viscosity model provided the best fit for the experimental data once it has the capability of handling Newtonian regions of shear-thinning fluids at low and high shear rates:

$$\eta_{FL} = \eta_{\infty} + \frac{\eta_0 - \eta_{\infty}}{\left(1 + m\dot{\gamma}^n\right)} \qquad (1)$$

Here, η_{FL} is the apparent viscosity of the floc suspension, η_0 and η_{∞} are viscosities of Newtonian regions for low and high shear rates, respectively, $\dot{\gamma}$ is the shear rate and m and n are correlation coefficients.

The experimental viscosity curves with the corresponding Cross fits are depicted in Fig. 3. As would be expected, the floc suspensions show a yield stress that increases with biomass concentration. Unfortunately, it was not possible to collect enough data at low stresses to accurately determine the yield stress. In the flow region, the behaviour of these suspensions can be divided into two different categories. Above a biomass concentration of 59 g.dm^{-3}, the behaviour is a typical one with a first Newtonian plateau at low stress, followed by a shear-thinning region and a second Newtonian plateau at very high stresses (in these cases, the Cross model was fit only to the data in the flow region, as can be seen from the Figure). Below a biomass concentration of 59 g.dm^{-3}, the behaviour is essentially Newtonian.

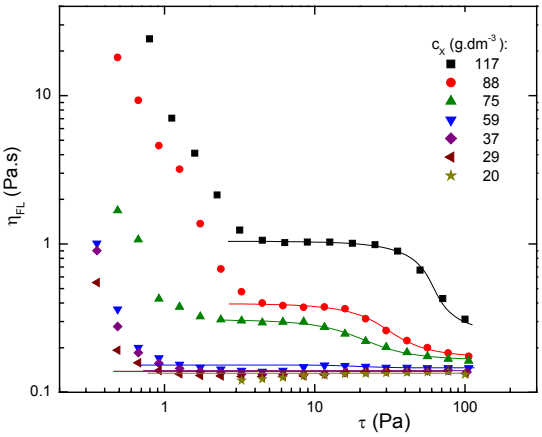

Figure 3. Viscosity curves as a function of shear stress, τ, for different biomass concentrations, c_X. The lines represent the fittings using the Cross model. All measurements were performed at 30 °C.

Several authors have reported on the viscoelastic behaviour of yeast suspensions. Labuza et al. [10] demonstrated the shear-thinning behaviour of baker's yeast (*S. cerevisiae*) for shear rates in the range of 1 to 100 s^{-1} and for yeast concentrations above 10.5 % (w/w) and used the power-law model successfully to fit the data. More recently, Mancini and Moresi [11] also measured the rheology of baker's yeast using different rheometers in the concentration range of 25 to 200 g.dm^{-3}. While Haake's rotational viscometer confirmed Labuza´s results on the shear-thinning character of the yeast suspension, dynamic stress rheometry revealed a definite Newtonian behaviour. They attributed this discrepancy to a lower sensibility of the Haake viscometer in the range of viscosity tested (1.5-12 mPa.s). Speers et al. [12] used a controlled shear-rate rheometer with a plate-cone system to measure the viscosity of suspensions of flocculating and nonflocculating strains of *S. cerevisiae* and *S. uvarum*. They derived a "cell floc" model, which used the well-known Bingham model for the description of viscoplastic flow behaviour of cell suspensions:

The viscosities observed were in the range of 4.6 to 26.9 mPa.s for shear rates up to 1000 s^{-1} and concentration of 2.5 x 10^9 cells/ml (corresponding to c_X of about 50 g.dm^{-3}), measured at 15 °C. Moreover, they discovered an important fact: the flocculating strains had much higher

apparent viscosity and yield stress than non-flocculating ones, which is an indication that the flocculation mechanism of yeast cells has a significant impact on their flow behaviour. On the other hand, the shear rate strongly affects floc structures.

It is hardly possible to compare quantitatively the viscosities of yeast suspensions due to its strong relations with the flocculating properties of the yeast strain, as well as to the effect of other factors reported e.g. in the work of Speers et al. [13]. From all the factors, however, cell concentration and shear rate have the most important effect. It can be concluded from the available literature that yeast suspensions below certain concentrations display a Newtonian behaviour, whereas above this limit the flow behaviour changes to either shear-thinning or viscoplastic.

4 RELATION OF ALR HYDRODYNAMICS WITH RHEOLOGY

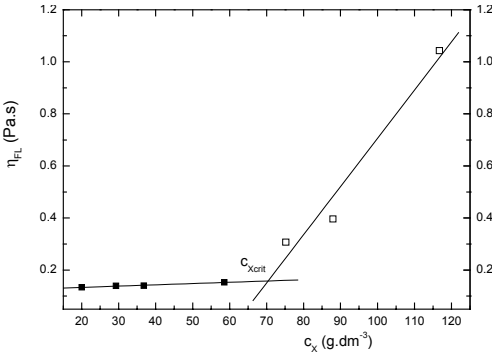

Figure 4. Dependence of viscosity of floc suspension on biomass concentration. c_{Xcrit} is critical value of biomass concentration determined from an intersection of two regression lines.

Figure 4 shows the dependence of zero-shear-rate apparent viscosity of floc suspension on biomass concentration. It clearly demonstrates a dramatic increase of viscosity at c_X in the interval of 60 to 75 g.dm^{-3}. In order to determine accurately the point of the strongest increase of viscosity, the intersection of two regression lines for low and high ranges of biomass concentration was calculated and a value of 70.4 g.dm^{-3} was found, which is in a very good agreement with the critical biomass concentration determined from the hydrodynamic measurements (only a 3.7 % difference). This finding explains the strong decrease observed in circulation velocity when the biomass concentration increases beyond its critical value. If biomass accumulation continues, the viscosity increases and net circulation slows down, which has a significant negative impact on most of the important phenomena of the bioprocess (mass and heat transfer, mixing, distribution of solid phase, kinetics) and increases the risk of bioreactor stalling. The reasons for this dramatic increase in viscosity above a critical concentration of biomass are not immediately apparent and will be the subject of future work.

5 CONCLUSIONS

The hydrodynamic analysis of a high cell density airlift bioreactor during the alcoholic fermentation showed dramatic changes in circulation velocity. Gas flow rate and especially biomass concentration were found to have the strongest effect on the bioreactor hydrodynamics. Measurements of liquid circulation velocity showed the existence of a critical value of biomass concentration at which a dramatic deceleration of net liquid flow appeared with increasing amount of biomass. Rheological analysis revealed strong changes in the viscosity of yeast floc suspensions during fermentation and proved that the breakpoint at the velocity curve corresponds very well to a point of the highest increase of suspension viscosity. From the practical point of the use of airlift bioreactor as a high cell density system, this means that the bioreactor should operate below the critical biomass concentration to ensure a safe operation in desirable circulated bed flow regime. The ALR can be operated with higher solid loadings up to a maximal biomass concentration; however, this would have a negative effect on transport phenomena, mixing and solid distribution in the bioreactor. Such information on hydrodynamics in the airlift bioreactor can be used *a priori* or during the bioprocess to optimise operational parameters in order to avoid the occurrence of undesirable bioreactor stalling and to maximize the process productivity.

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